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RAPID COMMUNICATION

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Cross-linking of plant cell walls with dehydrated fructose by smoke-heat treatment

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Introduction

In the primary wall of monocotyledons, the cellulose microfibrils could be cross-linked with xylans by hydrogen bonds, but in dicotyledons, xyloglucans cross-link the microfibrils.[1] We believe that the balanced dimensional stabilization of the wall is further attained by the formation of cross-linkages of both parenchymatous cells and xylem with dehydrated free sugars. The formation of cross-linkages could be reflected in both the dimensional stabilization and the increased elastic modulus, which occur in a similar manner after treating wood with formaldehyde.[2,3] Ketose (fructose and sorbose) and glucose could be easily dehydrated to 5-hydroxymethyl-furfural and levoglucosan and be cross-linked in the presence of acid at temperatures higher than 80°C by further dehydration.[4] In the plant cell walls, some of the cell wall components or the pyrolytic products of smoke might catalyze their dehydration. Thus, these dehydrated derivatives could be further dehydrated to cross-link and condense to humines, with which fructose and wall components are known to be highly reactive.[5] This practical recycling of plant cell wall by-products could lead not only to a new era of wood production but also to decrease the CO_2 emissions that worsen global warming.

Experimental

Garlic bulbs were obtained at a market in Japan. Twenty four year-old oil palms were cut in Malaysia and their stems were used after they were left on the ground for 2 weeks. For preparation of "complementary" palm stem specimens, each specimen was extracted with distilled water under reduced pressure and thoroughly washed with water and then submerged in the exogenous sugar solution to make its content 5% of the total dry weight of each piece ($5 \times 3 \times 1$ cm). Next, the specimens were brought to 80% water content and smoke-heated at 110°C for 30 h.

After filter paper was attached to the transversely cut garlic bulb or palm-stem waste, the paper was stained for fructose using the naphthoresorcinol method.[6] Free sugar was subjected to paper chromatography with 1-propanol/ethyl acetate/water (3:2:1, v/v), after extraction of garlic or palm tissue with 70% ethanol and its mild acid hydrolysis with 0.01 M trifluoroacetic acid (pH 2.0) at 100°C for 15 min. Total sugar in each fraction was determined by the phenol–sulfuric acid method.[7]

For determination of swelling levels, ten pieces ($1 \times 1 \times 1$ cm for garlic bulb and $3 \times 3 \times 1$ cm for palm stem at different regions) excised after treatment were used as specimens from smoke-heated samples. Specimens were brought to equilibrium with the relative vapor pressures of 22.2%, 57.3%, 75.3%, or 100% in the presence of saturated solutions of potassium acetate, sodium bromine, sodium chloride, or water in a desiccator at 25°C for 3 weeks. A powdered stem specimen (0.4 mg) was analyzed by pyrolytic gas chromatography-mass spectrometry (GC-MS) on a TC-1 glass capillary column (0.25×30 m). To determine elasticity, ten stem pieces ($3 \times 3 \times 1$ cm) were tested using a Shimadzu

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Computermatic grading machine for bending under a central point load.

Results and discussion

Garlic (*Allium sativum*) bulbs contain large quantities of fructan (inulin), which is easily hydrolyzed to fructose and fructo-oligosaccharides estimated as over 5% fructose content on dry weight (Fig. 1A, B).[8] The paper chromatographic pattern of the hydrolyzate was shown after smoke heating for 1 h and 24 h. When garlic bulbs were placed in the oven and smoke-heated at 90°C, the bulbs smoke-heated for 24 h showed fewer signs of swelling after being allowed to equilibrate with a range of relative vapor pressures (Fig. 1C). The sum of the tangential, radial, and longitudinal swelling varied widely for the air-dried specimens, but the variation was less for the smoke-heated specimens.

The distribution of fructose on the transversely cut stem of waste oil palm (*Elaeis guineensis*) stems is shown in Fig. 2A. The fructose content was relatively higher in the inner region, confirming claims made in a previous report by Mansor and Ahmad.[9] The fructo-oligosaccharides are quickly degraded to fructose and glucose when the palms are cut down, and can also be degraded to fructose and glucose by mild acid treatment (Fig. 2B). The amounts of free sugars were around 5% fructose and 1% glucose and inositol by dry weight. The smoke heat treatment caused a marked decrease in the amount of free sugars.

In order to clarify the effects of free sugars on the dimensional stabilization of wasted palm stems, we prepared stem specimens in which the free sugars had been replaced with exogenous sugars, and we analyzed the specimens. The specimens were extracted with distilled water under reduced pressure by changing the water many times (the piece of stem was washed thoroughly with water) and submerged in a fructose solution, a sorbose solution, and a glucose solution to induce a free sugar content of 5% of the total final dry weight. We called this the complementary specimen. By smoke heating after replacing the endogenous free sugars with exogenous sugars, the stems showed fewer signs of swelling after being allowed to equilibrate with a range of relative vapor pressures (Fig. 2C). The sum of the tangential, radial, and longitudinal swelling varied with the type of sugar used in the replacement. Smoke heating caused an increase in the Young's modulus and maximum stress (Fig. 2D), so that the mechanical properties of the stem specimens were close to those of the balsa.[10] Because fructose and sorbose are easily dehydrated to 5-hydroxymethyl-furfural and levoglucosan, leading to cross-linking by further dehydration in the presence of acid at temperatures higher than 80°C,[11] the replacement with the ketose (fructose and sorbose) was more effective than that with the aldose (glucose) in dimensional stabilization.

Pyrolytic GC-MS of native stems (not fresh but without heating; partially air-dried stem material) revealed a broad peak between 120° and 130°C that consisted of a number of minor peaks (Fig. 2E, left panel). A peak at 124°C corresponded primarily to 5-hydroxymethyl-furfural (*m/z* 127, 126, 109, and 97), and the peak at 125.5°C represented levoglucosan (*m/z* 144, 126, 98, and 73).[12] Neither the smoke-heated stem (see Fig. 2E, left "After") nor the water-extracted stem (see Fig. 2E, right "Sugar free") gave rise to any peaks between 120° and 130°C. After replacing the water-soluble components (the piece of stem was washed thoroughly with water) with fructose, the stem specimen produced the same broad peak between 120° and 130°C (see Fig. 2E, right panel).

For smoke heating, each wasted oil palm stem (40-cm diameter) was placed in an oven and smoke-heated at 200°C for 3 days over burning oil palm leaf waste. The temperature of the stem rose to 98°C at 3 cm from the cortex and to 80°C at the center of the 38-cm trunk. The water content of the stem after the 3-day treatment was about 50% of that of the stem without the treatment. The presence of bark around the stem during smoke heating was required for stabilization of the wall structures.[13] When the temperature was lower than 200°C or the duration of treatment was 48 h or less, the flat-grain planks bent and shrank after subsequent drying (Fig. 3A, right). Dimensional stabilization was observed to a greater degree when heating thinly sliced stems (Fig. 3B, C). The vertical-longitudinal-grain boards bent (Fig. 3B, center and right) and the vertical-tangential-grain boards shrank and cracked after heating (Fig. 3C, center and right). It is necessary to adjust the heating temperature and heating period for each plant cell wall by smoke heating.

The smoke arising from burning waste plant leaf and stem contains pyrolytic products including formic acid, phenolic substances, and soot, which could be introduced into the stem via the cut surface concomitant with the evaporation of water. In this way, smoking could contribute directly to the dimensional stabilization of the cell walls for garlic bulb and palm stem, although the general mechanism underlying this dimensional stability remains unknown. It should be noted that dimensional stabilization could not be achieved by steaming, such as by the kiln-drying method.[14]

Huge amounts of plant cell wall material are wasted worldwide, and the cell walls are eventually converted to carbon dioxide that is released into the atmosphere. Southeast Asia is one of the most highly productive places for biomass on earth. Malaysia and Indonesia are major producers of oil palms, which are harvested and replanted in 25-year cycles. Because no use has been devised for the oil palm stem waste, a huge amount of waste (about 8.5 million carbon tons per year in Malaysia and Indonesia) is either cut into pieces and placed atop soil to be degraded by bacteria and fungi or is burned for quick disposal. We believe that the proposed method is sustainable, although wooden materials including waste oil palm stems can be obtained without oil and may be used in other applications, such as replacements for conventional plastics.

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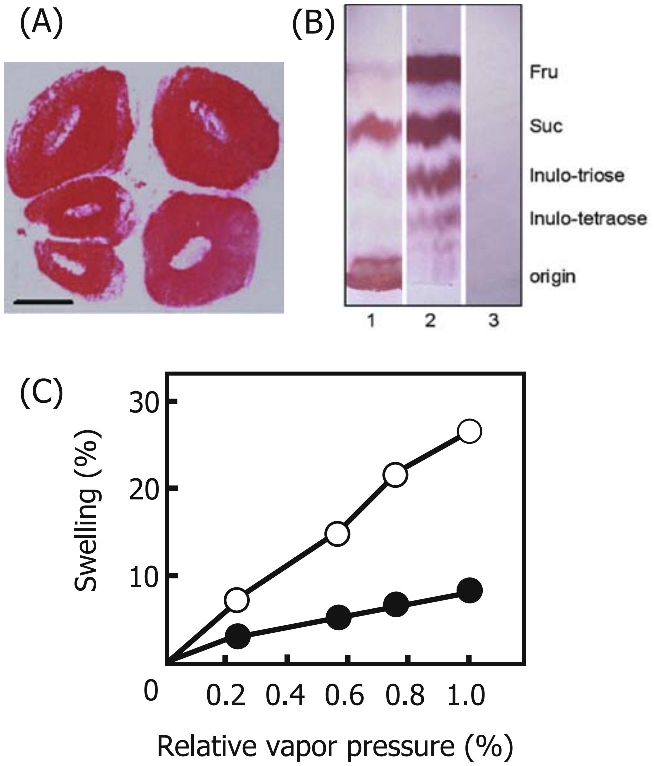

Fig. 1A–C. Sugar analysis of a garlic bulb. **A** In situ fructose staining of a garlic bulb. *Bar* 1 cm. **B** Soluble sugar analysis of the garlic bulb: *1*, bulb tissue attached; *2*, bulb tissue attached after smoke heating for 1 h; *3*, bulb tissue attached after smoke heating for 24 h. *Fru*, Fructose; *Suc*, sucrose. **C** Swelling of garlic bulbs after smoke heating. *Filled symbols*, smoke-heated garlic bulbs; *open symbols*, air-dried garlic bulbs. The values represent the means of five bulb samples with less than 8% variation

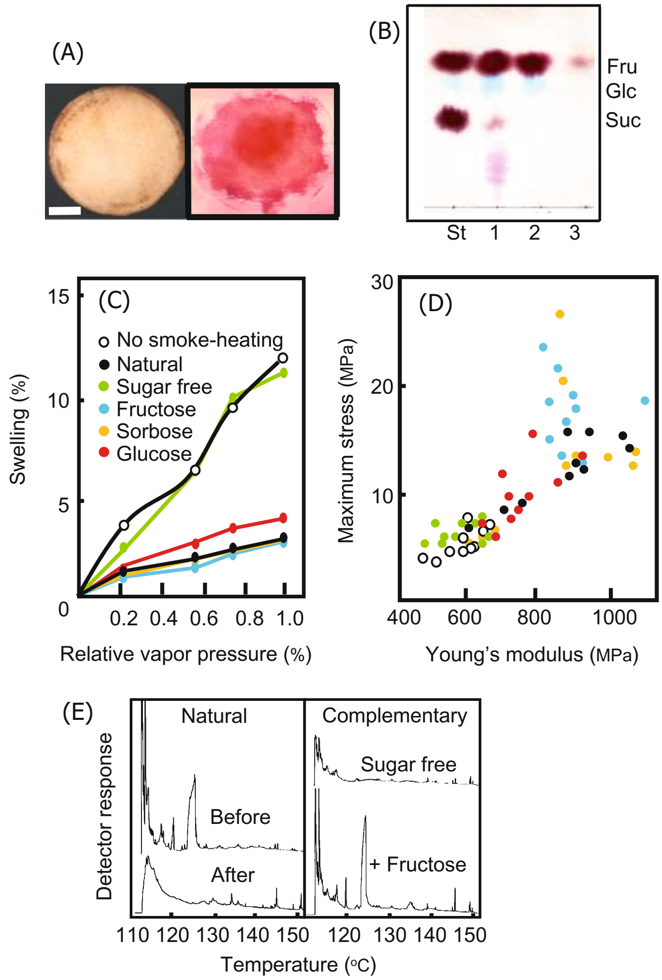

Fig. 2A–E. Sugar analysis of palm stem. **A** In situ fructose staining of a stem. A transversely cut stem (*left*) stained for fructose (*right*). *Bar* 10 cm. **B** Free-sugar analysis of palm stems: *St*, authentic sugars; *1*, soluble sugar; *2*, soluble sugar after mild acid hydrolysis; *3*, soluble sugar from smoke-heated stems; *Glc*, glucose. After paper chromatography, fructose was stained. **C** Swelling of the stems after smoke heating. The values represent the means of ten stem samples with less than 5% variation. **D** Maximum stress and Young's modulus of the stems after smoke heating. **E** Pyrolysis products of the palm stem. The panels show native stem specimens, before and after smoke heating (*left*); and complementary stem specimens, before and after replacement with fructose (*right*)

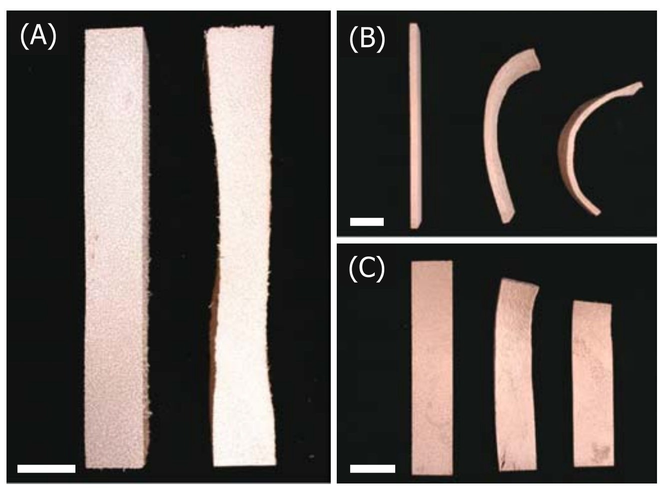

Fig. 3A–C. Effects of smoke heating on palm stems. **A** Flat-grain planks (radial, tangential, and longitudinal lengths: 30, 200, and 60 mm, respectively) after (*left panel*) and during (*right panel*) smoke heating. **B** Vertical-longitudinal-grain boards (radial, tangential, and longitudinal lengths: 150, 4, and 40 mm, respectively) after, during, and before smoke heating (*left to right*, respectively). **C** Vertical-tangential-grain boards (radial, tangential, and longitudinal lengths: 150, 30, and 4 mm, respectively) after, during, and before smoke heating (*left to right*, respectively). *Bars* **A** 3 cm, **B** 2 cm, **C** 4 cm

References

1. Hayashi T (1989) Xyloglucans in the primary wall. Annu Rev Plant Physiol Plant Mol Biol 40:139–168
2. Iwamoto Y, Itoh T, Minato K (2005) Vapor phase reaction of wood with maleic anhydride (II): mechanism of dimensional stabilization. J Wood Sci 51:601–606
3. Wang Y, Minato K, Iida I (2007) Mechanical properties of wood in an unstable state due to temperature changes, and analysis of the relevant mechanism III: effect of quenching on stress relaxation of chemically modified wood. J Wood Sci 53:94–99
4. Nomura T (2002) Dimensional stablilization of oil palm using smoke heat treatment. Proceedings of the 4th International Wood Science Symposium, Serpong, Indonesia, pp 36–42
5. Roxas ML (1916) The reaction between amino-acids and carbohydrates as a probable cause of humin formation. J Biol Chem 27:71–93
6. Forsyth WGC (1948) Colour reagents for the paper chromatography of sugars. Nature 161:239–240
7. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356
8. Suzuki M (1993) Science and technology of fructans. CRC, Boca Raton
9. Mansor H, Ahmad AR (1990) Carbohydrates in the oil palm and their potential use. J Trop Forest Sci 2:220–226
10. Forest Products Laboratory, US Department of Agriculture (1987) Wood handbook: wood as an engineering material. US Government Printing Office, Washington DC
11. van Dam HE, Kieboom APG, Delft HB (1986) The conversion of fructose and glucose in acidic media: formation of hydroxymethylfurfural. Starch 38:95–101
12. Anal MJ, Mok WSL (1990) Mechanism of formation of 5-(hydroxymethyl)-2-furaldehyde from D-fructose and sucrose. Carbohydr Res 199:91–109
13. Tejada A, Okuyama T, Yamamoto H, Yoshida M (1997) Reduction of growth stress in logs by direct heat treatment: assessment of a commercial-scale operation. Forest Prod J 47:86–93
14. Maeglin RR, Liu JY, Boone RS (1985) High-temperature drying and equalizing: effects on stress relief in yellow-poplar lumber. Wood Fiber Sci 17:240–253